EXHIBIT B

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A new SIV co-receptor, STRL33

The identification last year of chemokine receptors as fusion co-factors for HIV-1[1–6] has contributed significantly towards understanding HIV transmission and AIDS pathogenesis (see ref. 7 for a review). Because the experimental infection of rhesus macaques with simian immunodeficiency virus (SIV) and the resulting development of an AIDS-like illness is the best animal model for HIV disease in humans[8], identifying SIV co-receptors analogous to those used by HIV has obvious importance. We now report that STRL33, a chemokine receptor-like orphan receptor expressed in activated human lymphocytes and acting as a fusion co-factor with envelope glycoproteins (Envs) from HIV-1 strains of diverse tropisms[9], is a co-receptor for SIV.

The chemokine receptors CXCR4 and CCR5 are known to be important HIV-1 co-receptors for T-cell-line-tropic (TCL-tropic)[1] and macrophage-tropic (M-tropic) isolates[2–6], respectively. The chemokine receptors CCR2B and CCR3 can also act as co-receptors[5,6]. Although CCR5, but not CXCR4, CCR1, CCR2B, CCR3 or CCR4, can serve as a co-receptor for diverse strains of SIV[10–12], it is also clear that human peripheral blood mononuclear cells express an SIV co-receptor(s) distinct from CCR5[10].

First, we assessed SIV co-receptor activity of STRL33 and various chemokine receptors using the vaccinia-based cell fusion assay[13]. We tested cells expressing the Envs from SIV_{mac239} (TCL-tropic) and from SIV_{mac316} (M-tropic) for their ability to fuse with cells expressing CD4 and a single candidate co-receptor. STRL33 has potent fusion co-receptor activity with both SIV Envs (Fig. 1a). Consistent with previous reports[10–12], CCR5 functions with both Envs. We found no SIV_{mac} fusion co-receptor activity with CCR1, CCR2B, CCR3, CXCR4 (Fig. 1a) or CCR4 (not shown).

We next examined the ability of SIV_{mac239} to establish a productive infection in transfectants of human Jurkat T cells stably expressing STRL33 or CCR5. Both STRL33 and CCR5 render Jurkat cells permissive to SIV infection (Fig. 1b), whereas we observed no productive infection in the parental Jurkat cells (Fig. 1b) or in various transfected control cell clones (data not shown). The basis for the difference in replication kinetics in the Jurkat–STRL33 and the Jurkat–CCR5 cultures is unknown — contributing factors could be differences in the levels of co-receptor expression and the efficiencies of co-receptor function for SIV_{mac239}.

The discovery of individuals who are infected with HIV-1 despite being homozygous for an inactivating deletion in the CCR5 gene[14–16] shows that at least one receptor other than CCR5 can be important in HIV disease. Further investigation of STRL33 and other members of the co-receptor repertoire is thus critical for understanding the natural disease process, and may assume added significance if HIV-1 is placed under selective pressure by therapies designed to block a specific co-receptor. Further, the activity of STRL33 with SIV_{mac} has relevance to human AIDS beyond the general parallels between the human and simian systems, as SIV_{mac} is phylogenetically close to, and thought to be the immediate progenitor of, HIV-2 (ref. 17), a virus known to cause AIDS.

Together with our previous report that STRL33 functions with HIV-1 strains of diverse tropisms[9], our present findings with SIV demonstrate that STRL33 is active with a broader range of Envs than has been described for any of the co-receptors so far discovered. This attribute suggests that STRL33 will be of particular value in unravelling the structural determinants of interactions between Envs and co-receptors.

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•STRL33 is identical to Bonzo, one of the SIV co-receptors described by Deng *et al.* elsewhere in this issue[18]. See also N&V, pp. 230–231.

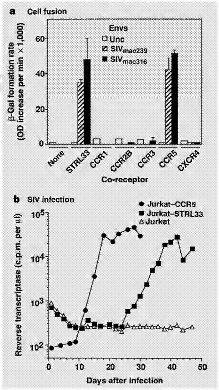

Figure 1 SIV co-receptor activity of STRL33, also referred to as Bonzo[18]. **a,** Vaccinia-based cell fusion assay (see citations in refs 1, 4 and 13 for methods). We transfected target cells (NIH 3T3) with plasmids containing the candidate co-receptor complementary DNAs linked to the bacteriophage T7 promoter and then co-infected them with vaccinia recombinant vTF7-3 encoding T7 RNA polymerase and vCB-3 encoding CD4. We co-infected effector cells (HeLa) with vCB-21R (containing the *Escherichia coli LacZ* gene linked to the T7 promoter) plus one of the following Env-encoding vaccinia recombinants: vCB-74 (SIV_{mac239}) or vCB-75 (SIV_{mac316}) (C. C. Broder and E. A. B., personal communication), or vCB-16 (nonfusogenic uncleaved Unc IIIB). We assessed cell fusion after 2 h by colorimetric assay of β-galactosidase activity in detergent cell lysates. **b,** Productive SIV_{mac239} infection of Jurkat cell transfectants. We prepared virus stock from HEK 293 cells transfected with SIV_{mac239} DNA and used it to infect Jurkat cell transfectants stably expressing STRL33 (ref. 9) or CCR5 (ref. 4) (as well as control Jurkat cells). We assessed virus production by measuring reverse transcriptase activity in the medium. The Jurkat-CCR5 infection was done in a separate experiment from other Jurkat infections.

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